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Role of T_H1/T_H2 Cytokines in HIV Infection

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INTRODUCTION

In 1986, Mosmann and Coffman analyzed a panel of murine $CD4^+$ T_H cell clones that revealed distinct patterns of cytokine production and effector functions. T_H1 cells secrete interleukin (IL)-2, tumor necrosis factor (TNF)-β, interferon (IFN)-γ and are the principal effector of cell-mediated immunity against intracellular microbes and of delayed-type hypersensitivity reactions. Murine T_H1 cells can also stimulate production of antibodies of the IgG2a class, which are effective at activating complement and opsonizing antigens for phagocytosis. T_H1 cells trigger phagocyte-mediated host defense and infections with intracellular microbes tend to induce T_H1-type responses. On the other hand, T_H2 cells produce IL-4 (which stimulates IgE and IgG1 antibody production), IL-5 (an eosinophil-activating factor), IL-10 and IL-13, which together with IL-4 inhibit some macrophage functions. Therefore, the T_H2 subset is mainly responsible for phagocyte-independent host defense, e.g. against certain helminthic parasites, which is mediated by IgE and eosinophils (Mosmann & Coffman 1989). During many strong immune responses these two effector pathways appear to be exclusive, because T_H1 and T_H2 cells are mutually inhibitory and/or self-stimulatory. Three cytokine activities are consistent with this: IFN-γ inhibits the growth of T_H2 cells, IL-4 preferentially stimulates the growth of T_H2 cells, and IL-10 suppresses the production of cytokines by T_H1 cells in response to antigen plus antigen-presenting cell (APC) (Gajewski & Fitch 1988, Mosmann & Moore 1991). In the absence of clearly polarizing signals, $CD4^+$ T_H cell subsets with a less differentiated cytokine profile

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than T_H1 or T_H2 cells, designated T_H0, usually arise (Street et al. 1990). T_H0 cells may dominate in the earliest stages of some immune responses and mediate intermediate effector functions, depending upon the ratio of cytokines produced and the nature of the responding cells.

For about 5 years, it looked as if such a dichotomic and cross-regulatory system might not be working in humans. Despite intense searching, several laboratories had failed to find evidence for the existence of distinct T_H1 and T_H2 subpopulations in healthy humans (Maggi et al. 1988, Paliard et al. 1988). Then, we looked at clones specific for peculiar antigens and succeeded. Most $CD4^+$ T_H cell clones specific for the excretory/secretory antigen(s) of the nematode *Toxocara canis* exhibited a T_H2 profile of cytokine secretion (production of IL-4 and IL-5), whereas the great majority of T_H cell clones specific for the purified protein derivative (PPD) from *Mycobacterium tuberculosis* generated from the same donors showed a clear-cut T_H1 profile (production of IL-2, IFN-γ and TNF-β) (Del Prete et al. 1991, Romagnani 1991). Similar data were obtained in other laboratories deriving T-cell clones specific for other antigens or expanding T cells infiltrating the target organs of patients suffering from different diseases (Del Prete et al. 1989, Wierenga et al. 1990, Brod & Hafler 1991, Kapsenberg et al. 1991, Maggi et al. 1991, Parronchi et al. 1991, Salgame et al. 1991, van der Hejiden et al. 1991, Yssel et al. 1991, Schlaak et al. 1992, Del Prete et al. 1993a). Subsequent findings have since supported the notion that T_H1 and T_H2 cells work functionally *in vivo* as well (Foulis et al. 1991, Hamid et al. 1991, Selmaj et al. 1991, Robinson et al. 1992, Field et al. 1993, Schandené et al. 1993, Cogan et al. 1994). Therefore, there is now a broad consensus on the existence of human $CD4^+$ T_H cells with cytokine patterns and functions that are comparable to murine T_H1 and T_H2 cells, although in humans the expression of some cytokines, such as IL-2, IL-6, IL-10 and IL-13, may be less restricted (Yssel et al. 1992, Del Prete et al. 1993b, Zurawski & de Vries 1994). To avoid oversimplification, however, it is opportune to emphasize that the profile of the T_H cell-mediated specific immune response is more complex that T_H1 and T_H2 patterns. Thus, T_H1 and T_H2 cells should not be regarded as the two functional subsets of $CD4^+$ T_H cells, but as extremely polarized forms of the heterogenous T_H cell-mediated effector response.

THE 'T_H1/T_H2 SWITCH' HYPOTHESIS IN HIV INFECTION

Following the demonstration of the existence of T_H1 and T_H2 subsets, it was shown that in certain infectious murine and human diseases, particularly in parasitic diseases, the T_H1 pattern of cytokines is usually associated with resistance to infection, whereas the T_H2 pattern is associated with progression of infection. The best example is non-healing forms of murine leishmaniasis, which represent strong, but harmful T_H2-dominated responses (Heinzel et al. 1989, Scott et al.

1989). Another example is lepromatous lepra where inappropriate T_H2 responses actively block disease-controlling T_H1 responses (Yamamura et al. 1991). These observations led Clerici & Shearer (1993a) to speculate that a switch from the T_H1 to the T_H2 cytokine phenotype may be important in the pathogenesis of disease progression in HIV-infection. This hypothesis was also based on two distinct sets of data on the immunology of HIV infection. First, Clerici et al. (1992) found that a large porportion of individuals exposed to HIV who tested negative for the virus, but a small percentage of unexposed or low risk subjects, showed evidence of HIV-specific cell-mediated immunity. In a second set of experiments, the same authors demonstrated that 50% of HIV-infected asymptomatic subjects initially had a good T-cell response; IL-2 was released after activation with influenza virus or with HIV peptides (Clerici et al. 1993a). These patients showed a gradual shift from T_H1 to T_H2 responses in the course of HIV infection. Loss of IL-2 responses to soluble antigens or HIV peptides was often accompanied by increased PHA-induced IL-4 and IL-10 production (Clerici et al. 1993b, 1994).

EXPERIMENTAL APPROACHES USED TO PROVE OR DISPROVE THE "T_H1/T_H2 SWITCH" HYPOTHESIS

In this review, the results of different experimental approaches designed to prove or disprove the T_H1/T_H2 switch hypothesis are reported.

Cytokine production in short-term PBMC cultures

In a first series of experiments we tried to reproduce the results reported by Clerici et al. (1993, 1994), by using short-term cultures of PBMC stimulated for 3 days with polyclonal activators, such as PHA or PMA plus anti-CD3 monoclonal antibody (mAb) or by a mixture of anti-CD2 and anti-CD28 mAb. The results obtained by stimulation of PBMC with PHA are reported in Table I. Production of IFN-γ was not significantly affected. Likewise, the production of both IL-4 and IL-10 by PBMC of HIV-infected patients was not increased, but rather was decreased, in comparison with IL-4 and IL-10 production by randomly matched HIV-seronegative healthy donors. More importantly, PBMC from HIV-infected patients with reduced numbers of circulating $CD4^+$ T cells produced lower amounts of both IL-4 and IL-10 in comparison with HIV-infected patients showing quite normal or only slightly decreased values of circulating $CD4^+$ T cells. Similar results were obtained by stimulating PBMC with PMA plus anti-CD3 mAb (Maggi et al. 1994) or anti-CD2 plus anti-CD28 mAb (data not shown).

In our opinion, however, the assessment of cytokine production by PBMC

TABLE I

Production of IFN-γ, IL-4 and IL-10 by fresh PBMC from HIV-infected individuals in response to stimulation with PHA

Subjects	No. of cases	Cytokine production by PBMC		
		IFN-γ (ng/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
HIV-seronegative	79	3.0 ± 0.6	48 ± 7	175 ± 62*
HIV-seropositive	83	4.0 ± 0.4	34 ± 4	50 ± 6*

PMBC (10^6/ml) from 79 age- and sex-matched HIV-seronegative and 83 HIV-seropositive subjects (33 belonging to Group II or III and 50 to Group IV of CDC classification) were cultured for 48 hours in RPMI 1640 medium-5% fetal calf serum in the presence of 1% (vol/vol) phytohemagglutinin (PHA). Cell-free culture supernatants were collected and assayed for their IFN-γ content by RIA (Centocor Inc., Malvern, PA) and for IL-4 and IL-10 content by ELISA (Quantikine R & D Systems, Minneapolis, MN and Assay Res. Inc., College Park, MD, respectively). For statistical analysis of the data the Student *t* test was used.

* $p < 0.02$.

stimulated with polyclonal activators does not represent the best approach to prove or disprove the T_H1/T_H2 switch hypothesis. First, PBMC consist not only of $CD4^+$ T cells but also of other cell types, such as macrophages, B-cells, NK-cells, and $CD8^+$ T cells, potentially inducible to cytokine production in response to the same stimulants. In addition, the proportions of the different cell types within the PBMC suspension may vary in different patients according to the phase of HIV infection. For example, $CD4^+$ T cells are decreased and all other cell types are relatively increased in the advanced phases of HIV-infection. To overcome this problem, purified $CD4^+$ T-cell suspensions were studied. Even under these experimental conditions, neither IL-4 nor IL-10 production were increased in HIV-infected individuals as compared to controls (data not shown). However, since IL-4 is produced in very small amounts in short-term cultures, its concentration is often under the detection limits of commonly used assays. Therefore, in subsequent experiments, cytokine production was assessed in long-term cultures of $CD4^+$ T cells, such as T-cell lines or T-cell clones.

Cytokine profile of PHA- or anti-CD3-induced $CD4^+$ T-cell lines and clones

Polyclonal $CD4^+$ T-cell lines were induced by stimulation with insolubilized anti-CD3 mAb of purified $CD4^+$ T cells from HIV-infected patients and HIV-seronegative healthy controls, followed by addition of IL-2. After 2 weeks, T-cell blasts (1×10^6/ml) were stimulated with PMA plus anti-CD3 mAb and the production of IL-4 and IFN-γ in the supernatant was assessed. Again, no increase in the production of either cytokine by $CD4^+$ polyclonal T-cell lines from HIV-infected versus controls was observed (Table II).

TABLE II

Cytokine production by anti-CD3-induced CD4+ or alloreactive T-cell lines obtained from HIV-infected individuals

	Cytokine synthesis			
	Anti-CD3-induced CD4+ T-cell lines		Alloreactive T-cell lines	
Patients (No. of T-cell lines)	IL-4 (pg/ml)	IFN-γ (ng/ml)	IL-4 (pg/ml)	IFN-γ (ng/ml)
Healthy controls (16)	231.6±90*	4.8±1.0	231±132	5.77±1.0
HIV-infected subjects (28)	36.0±10*	2.6±0.5	139±103	7.82±0.5

Alloreactive T-cell lines were derived from PBMC of 14 HIV-infected individuals (6 belonging to Group II or III and 8 to Group IV of CDC classification) and from 8 seronegative controls following stimulation for 5 days with irradiated PBMC of 2 healthy subjects. CD4+ T-cell lines were obtained from purified CD4+ T cells of the same subjects stimulated with insolubilized anti-CD3 antibody for 5 days. T-cell blasts were then expanded with IL-2 for an additional 9 days and stimulated for 48 hours with PMA and anti-CD3 antibody. Cell-free supernatants were assessed for IL-4 and IFN-γ, as described (Maggi et al. 1988).
* $p < 0.05$.

T-cell clones were also generated from the peripheral blood of 9 HIV-infected patients and 9 HIV-seronegative healthy controls according to a cloning technique allowing the clonal expansion of virtually every single T-cell (naive, memory, resting, activated) (Moretta et al. 1983). A total of 344 CD4+ T-cell clones were obtained from HIV-seronegative donors, whereas CD4+ T-cell clones derived from the HIV-infected patients totalled 404. All clones were assessed for IL-4 and IFN-γ production upon stimulation with PMA plus anti-CD3 mAb. The results of these experiments are summarized in Fig. 1. The proportions of CD4+ T-cell clones inducible to IFN-γ production were not significantly different in the two groups of subjects, whereas the proportions of CD4+ T-cell clones inducible to IL-4 production were significantly reduced in HIV-infected patients in comparison with controls (Maggi et al. 1994a). This reduction was due to the preferential depletion of IL-4-producing CD4+ T cells in patients with very low numbers of circulating CD4+ T cells (< 400/μl).

Cytokine profile of T-cell clones derived from skin biopsy specimens

The next approach was to assess the cytokine secretion profile of T-cell clones derived from skin biopsy specimens obtained from 4 HIV-infected patients; 3 of them had Kaposi's sarcoma, the 4th being a volunteer without skin diseases. As controls, skin biopsy specimens derived from 8 volunteers (4 without any skin disease and 4 suffering from atopic dermatitis) were used. Skin specimens were cultured *in vitro* with IL-2 in order to expand T cells already expressing IL-2

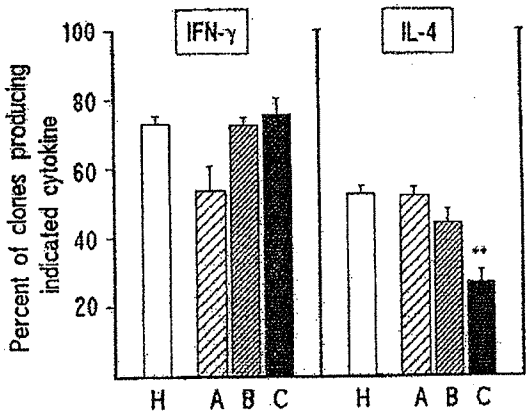

Figure 1. Cytokine production in response to stimulation with PMA plus anti-CD3 antibody of $CD4^+$ T-cell clones derived from PBMC of HIV-infected individuals. $CD4^+$ T-cell clones were derived by PHA stimulation (Moretta et al. 1983) of PBMC from 9 HIV-seronegative (H) and 9 HIV-seropositive individuals. Three of them had > 500 $CD4^+$ T cells/μl (A), 3 < 500 and > 200 $CD4^+$ T cells/μl (B) and 3 < 200 $CD4^+$ T cells/μl (C). T-cell blasts (10^6/ml) from each $CD4^+$ T-cell clone were stimulated for 48 hours with PMA (10 ng/ml) and anti-CD3 mAb (100 ng/ml) and cell-free supernatants assessed for their cytokine content. IL-4 and IFN-γ were quantitated by RIA and ELISA, as reported in Table I. Cytokine levels 5 SD over the mean levels of controls (supernatants derived from stimulation of irradiated feeder cells alone) were regarded as positive (** $p < 0.0005$).

receptor. Growing T cells were then cloned with PHA under limiting dilution conditions, as described (Del Prete et al. 1993a), so that deriving T-cell clones mainly represented the progenies of *in vivo*-activated T cells. Overall, 609 T-cell clones were generated from skin biopsies of the 4 HIV-infected patients, 330 from the 4 healthy subjects and 341 from the 4 patients with atopic dermatitis. The great majority of clones generated from both healthy subjects and patients with atopic dermatitis were $CD4^+$ (86% and 73%, respectively), the others being $CD8^+$, whereas the great majority of skin-derived clones (89%) in HIV-infected patients were $CD8^+$ and only 11% were $CD4^+$. The profiles of cytokine production by both $CD4^+$ and $CD8^+$ clones in response to stimulation with PMA plus anti-CD3 mAb are summarized in Table III. The proportions of IFN-γ-producing clones from the skin of HIV-infected patients and healthy individuals were not significantly different, whereas the proportion of IFN-γ-producing clones from patients with atopic dermatitis was significantly reduced. In contrast, the proportions of $CD4^+$ IL-4-producing clones were significantly higher in both HIV-infected patients and patients with atopic dermatitis in comparison with healthy individuals. Surprisingly, the proportions of IL-4-producing $CD8^+$ T-cell clones generated from the skin of HIV-infected individuals were also significantly higher

TABLE III

Cytokine production by T-cell clones obtained from the skin of HIV-seropositive and HIV-seronegative individuals

Source of T-cell clones	Phenotype (No.) of T-cell clones	No. (%) of T-cell clones producing: IFN-γ	IL-4
HIV-seronegative (11)			
Nonatopic (4)	$CD4^+$ (285)	167 (59)[a]	71 (25)[c]
	$CD8^+$ (45)	27 (60)	12 (27)[f]
Atopic (7)	$CD4^+$ (351)	169 (48)[b]	173 (49)[d]
	$CD8^+$ (92)	64 (69)	23 (25)
HIV-seropositive (4)			
	$CD4^+$ (67)	33 (49)	29 (43)[e]
	$CD8^+$ (542)	326 (60)	201 (37)[g]

Skin biopsies were obtained from 4 HIV-infected patients (1 asymptomatic and 3 with Kaposi's sarcoma) and from 11 HIV-seronegative individuals (7 with atopic dermatitis and 4 healthy subjects). Biopsy specimens were cultured for 9-12 days in IL-2-conditioned medium as reported (Del Prete et al. 1993a). Growing T blasts were then cloned with PHA and IL-2 in the presence of irradiated feeder cells. T-cell blasts of each clone (10^6/ml) were stimulated for 48 hours with PMA (10 ng/ml) plus anti-CD3 (100 ng/ml) mAb. Cytokine production was assessed in the cell-free supernatants with calibrated RIA or ELISA, as described (Maggi et al. 1988).

[a] vs [b]: $p < 0.01$; [c] vs [d]: $p < 0.001$; [e] vs [f]: $p < 0.001$; [g] vs [h]: $p < 0.05$.

in comparison with both healthy subjects and patients with atopic dermatitis (Table III) (Maggi et al. 1994a).

The demonstration of noticeable proportions of $CD8^+$ T cells showing a T_H2-like profile of cytokine secretion in the skin of HIV-infected patients was supported by another series of experiments performed in 2 HIV-infected individuals suffering from an adult-onset Job's-like-syndrome (Paganelli et al. 1993, Raiteri et al. 1993). Both patients had recurrent skin and sinopulmonary infections and showed very high serum IgE levels (> 3000 IU/ml) and eosinophilia (> 500/μl). Of note, both patients showed marked depletion of circulating $CD4^+$ T cells (< 50/μl), but normal or slightly elevated numbers of circulating $CD8^+$ T cells. Most $CD3^+$ T-cell clones derived from the peripheral blood or the skin of the 2 patients according to the procedures reported above (Moretta et al. 1983, Del Prete et al. 1993a) were $CD4^-CD8^+$ or $CD4^-CD8^-$. Virtually none of the $CD4^-CD8^+$ or $CD4^-CD8^-$ clones produced IFN-γ and they all exhibited reduced cytolytic activity, produced large amounts of IL-4 and IL-5 and provided helper function for IgE synthesis. Interestingly, most $CD4^-CD8^-$-T_H2-like noncytolytic clones expressed mRNA for CD8α chain (Maggi et al. 1994b). These cells strongly resemble the $CD4^-CD8^-$ cells resulting from switching of murine $CD8^+$ cells incubated *in vitro* with high IL-4 concentrations (Erard et al. 1993), suggesting they might result from an *in vivo* switching into T_H2-like cells of $CD8^+$, originally

T_H1-like and cytolytic, T cells conditioned by high IL-4 concentrations present in the microenvironment.

Antigen-specific T-cell clones

To solve the apparent contradiction between the results obtained with clones derived by PHA-stimulation of single T cells (reflecting the expansion of all types of T cells) and those derived from IL-2-conditioned T-cell lines (mainly reflecting the expansion of T cells already activated *in vivo*), we then looked at the cytokine secretion profile of antigen-specific T-cell clones derived from the PB. This cloning system allows one to explore the differentiation of memory T lymphocytes under the collaborative influence of APCs and other cell types, thus probably reflecting the microenvironmental conditions that are usually operating *in vivo* better than the cloning system based on PHA stimulation. T-cell clones specific for *Toxoplasma gondii* (Txpl) antigen(s) were derived from the blood of 3 HIV-seronegative healthy donors and 3 HIV-infected individuals according to a cloning technique previously described in detail (Del Prete et al. 1991, Parronchi et al. 1991). A total of 149 Txpl-specific $CD4^+$ T-cell clones were obtained from the 3 HIV-seronegative subjects, whereas Txpl-specific $CD4^+$ T-cell clones derived from HIV-seropositive subjects numbered 179. When assessed for their cytokine secretion profile in response to PHA, 40% of Txpl-specific clones from HIV-seronegative subjects showed a clear-cut T_H1 profile (production of IFN-γ and TNF-β, but not IL-4), whereas the remaining 60% exhibited a mixed (T_H0) phenotype (Table IV). In contrast, virtually all $CD4^+$ Txpl-specific T-cell clones generated from HIV-seropositive donors produced not only IFN-γ and TNF-β, but also IL-4, IL-5 and IL-10, suggesting a general shift towards the T_H0 profile (Table

TABLE IV

Cytokine production by Toxoplasma g.-specific T-cell clones derived from HIV-seropositive and HIV-seronegative donors

Source of T-cell clones	No. of $CD4^+$ T-cell clones	Percent of clones producing				
		IFN-γ	TNF-β	IL-4	IL-5	IL-10
HIV-seronegative (n = 3)	149	100	92	60	36	65
HIV-seropositive (n = 3)	179	97	96	89*	72*	92*

PBMC from 3 HIV-seronegative and 3 HIV-seropositive subjects were stimulated for 6 days with *Toxoplasma gondii* extract followed by IL-2. Growing T blasts were then cloned with PHA and IL-2 in the presence of irradiated feeder cells. T-cell clones specific for Toxoplasma g. antigen(s) were identified on the basis of their proliferative response to Ag under MHC-restricted conditions. For induction of cytokine secretion, T-cell clones (10^6 cells/ml) were stimulated for 36 hours with PHA. Cytokines were measured as previously described (Del Prete et al. 1991).

* $p < 0.0001$.

IV). T-cell clones specific for PPD were also generated from the blood of another HIV-seropositive subject and a healthy individual since PPD has been found to act as a T_H1-inducing antigen in healthy individuals (Del Prete et al. 1991). As expected, the great majority (80%) of PPD-specific T-cell clones derived from the HIV-seronegative subject had a clear-cut T_H1 profile, the other being T_H0; in contrast, the majority of PPD-specific clones (71%) derived from the HIV-seropositive donor exhibited a mixed (T_H0) phenotype and only a minority (29%) were T_H1. Virtually no T_H2 clones specific for Txpl or PPD in either HIV-infected donors or HIV-seronegative controls were observed (Maggi et al. 1994a).

Taken together, these data suggest that $CD4^+$ T cells from HIV-infected individuals activated *in vitro* with PPD or Txpl antigens develop into T-cell clones that retain the ability to produce T_H1-type cytokines like their counterparts from HIV-seronegative subjects, but also exhibit a greater ability to produce T_H2-type cytokines. The reason for this difference is at present unclear. The fact that such a property cannot be revealed by expanding T cells from HIV-seropositive subjects in a different microenvironment (e.g. stimulation of single T cells with PHA in the absence of autologous accessory cells) suggests a possible role for APCs or other cells present in bulk PBMC cultures at the time of antigen stimulation. Another possibility is that at least a proportion of cells producing T_H2 cytokines are lost following stimulation with PHA, due to apoptosis or other HIV-related cell-death mechanisms. It has indeed been shown (and confirmed again in this study) that the cloning procedure based on PHA stimulation of single T cells usually results in significantly lower cloning efficiencies in HIV-infected patients compared to HIV-seronegative healthy individuals (Margolick et al. 1985, Maggi et al. 1987).

Changes in serum IgE levels in HIV-infected individuals

The *in vitro* assays described above can reveal differences in the cytokine profile of $CD4^+$ T-cell clones between HIV-infected subjects and HIV-seronegative controls or among HIV-infected subjects in different phases of HIV-infection, but they do not allow detection of possible changes in the cytokine secretion profile during the course of HIV-infection in the same subjects. Longitudinal studies performed with sophisticated *in vitro* techniques are indeed difficult to perform. Nevertheless, a recent study based on cloning of CD45RO (memory) T cells, obtained from the same donor at different time intervals after serodiagnosis and stored in a frozen state, revealed shifting towards the T_H0 secretion profile with the progression of infection (Meyaard et al. 1994).

A more simple approach to longitudinal study of HIV-seropositive subjects may be to assess possible changes of serum IgE levels during the course of infection. Elevated IgE levels are indeed common in patients with allergic disorders and helminthic infections and appear to be related to the occurrence of T_H2-

type responses against common environmental allergens or helminth components, respectively (Del Prete et al. 1991, Parronchi et al. 1991). Thus, a shifting from the T_H1 to the T_H2 profile of cytokine secretion in HIV-infected patients would result in enhanced IgE synthesis in response to encountered antigens and, therefore, in the increase of serum IgE levels.

To assess this possibility, IgE levels were quantitated in the sera taken from 99 HIV-infected patients at the time of serodiagnosis, as well as 4 and 8 years later. As shown in Fig. 2, a significant and progressive reduction in the mean value of circulating $CD4^+$ T cells occurred in this group of patients, but the mean serum IgE level remained unchanged. There was, however, a different behavior of serum IgE concentrations at the individual level. Sixty-one patients, who exhibited low IgE levels at the beginning, showed no significant change in the two subsequent determinations; 12 patients showed transient elevation after 4 years, whereas 17 patients revealed increased IgE levels only after 8 years. Finally, 9 patients, who had increased IgE levels at the beginning, showed progressive reduction in the two subsequent determinations.

Thus, in a noticeable proportion of HIV-infected patients (total 38%) enhanced IgE synthesis in the early, intermediate or advanced phase of infection may be revealed; however, in the majority of patients serum IgE concentrations remain low during the entire course of infection. This finding strongly argues against the possibility that a T_H1/T_H2 switch occurs, at least in the majority of patients. It does not exclude, however, the possibility of an increased production of T_H2 cytokines, as suggested by the results obtained with antigen-specific T-cell clones. In fact, T_H0 cells usually do not provide help for IgE synthesis because the IgE-inducing capacity of IL-4 is antagonized by the activity of IFN-γ produced by the same cells (Del Prete et al. 1988).

PREFERENTIAL HIV REPLICATION IN $CD4^+$ T-CELL CLONES PRODUCING T_H2-TYPE CYTOKINES

In another series of experiments, we have also looked at the effect of HIV-infection on T-cell clones with established cytokine profile derived from HIV-seronegative individuals. Fifty-two T-cell clones specific for different antigens (PPD, tetanus toxoid or allergens) were infected *in vitro* with HIV, as previously described (Macchia et al. 1991, 1993). Two to 3 weeks later, the presence of DNA provirus in T-cell clones was assessed by semiquantitative PCR (Carbonari et al. 1993) and viral replication was detected by measurement of p24 antigen (p24 Ag) in their supernatants. All clones expressed DNA provirus, but only a proportion of them showed detectable p24 Ag production. In particular, p24 Ag was detected in the supernatant of all 11 T_H2 clones and in 22 out of 33 T_H0 clones, but in none of the 8 T_H1 clones tested. The results relative to some representative clones are reported in Table V. These findings, which are in agreement with recent results

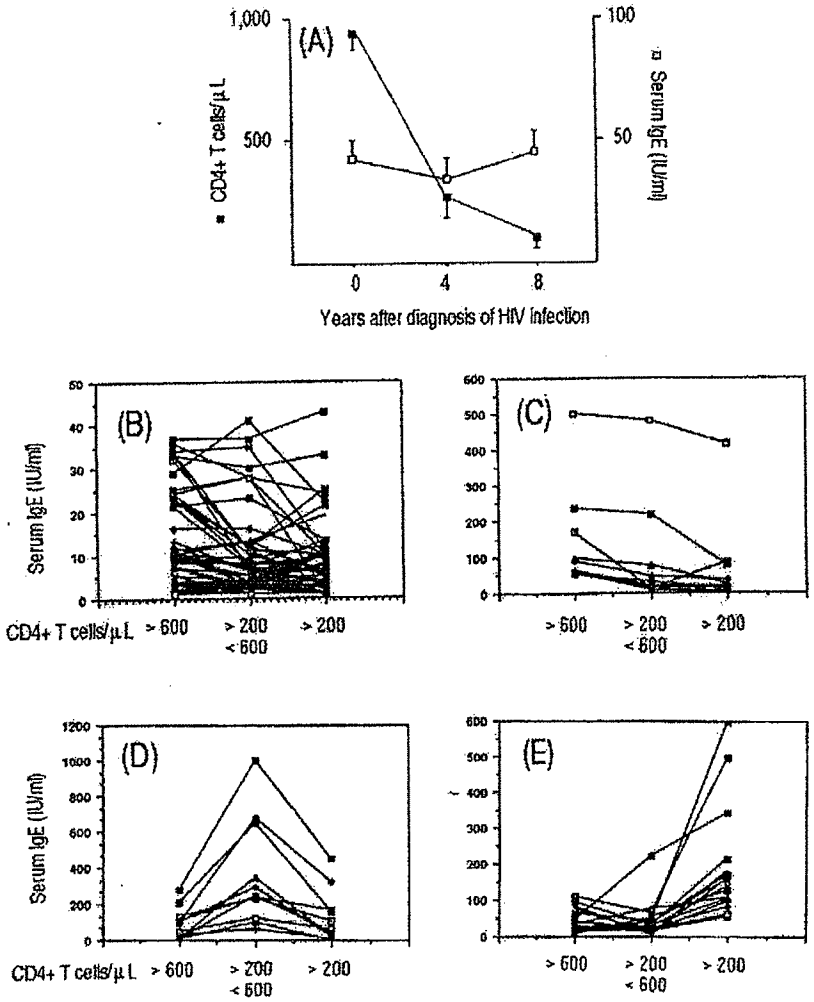

Figure 2. Follow-up of IgE serum levels in HIV-infected individuals. (A) The numbers of $CD4^+$ T cells/μl ($\pm$) were assessed in 99 HIV-seropositive individuals at different time intervals from diagnosis. Mean values ($\pm$SE) are reported. (B-D) In the same patients, the behavior of serum IgE was also evaluated in respect of the progressive fall of circulating $CD4^+$ T cells. In the majority of them (61 out of 99) IgE serum levels remained in the normal range irrespective of the progressive reduction of $CD4^+$ T cells (B). Nine patients, with elevated serum IgE at the diagnosis, showed a progressive decrease of IgE (C). In the other 29 patients an increase of IgE levels was observed at an intermediate (D) or the final (E) phase of their $CD4^+$ depletion process.

obtained by Vyakarnam et al. (1994), suggest that T-cell clones producing T_H2 cytokines more efficiently support viral replication than T-cell clones producing T_H1 cytokines alone. They may also be consistent with the data reported by Mackewics & Levy (1992) and by Brinchmann et al. (1990), suggesting nonlytic suppression of virus replication mediated by soluble factor(s) released by $CD8^+$ T cells. Indeed, since T_H1 $CD4^+$ cells exhibit a cytokine profile similar to that of $CD8^+$ T cells, it cannot be excluded that the same factor(s) is also responsible for the lower efficiency of these cells in supporting HIV replication. The nature of the $CD8^+$ T cell-derived suppressive factor (Brinchmann et al. 1990, Mackewics & Levy 1992) is still unknown, even though its activity does not seem to be attributable to any of the already known cytokines. However, IFN-γ, whose inhibitory activity against HIV-infection is well known (Wong & Goeddel 1986), may at least in part contribute to this suppressive effect. In more recent experiments, we have indeed shown that the addition of anti-IFN-γ receptor antibody may trigger p24 Ag production by highly purified $CD4^+$ T cells from some HIV-infected patients (Maggi et al. 1994a). In the same or in some other patients the addition of IL-4 and/or IL-10 also triggered p24 Ag secretion. Based on these data, it is reasonable to suggest that both autocrine and paracrine IFN-γ produc-

TABLE V

Cytokine and p24 Ag production by T_H1-, T_H0- and T_H2-like $CD4^+$ T-cell clones infected in vitro *with HIV*

T-cell clones	Cytokines produced by T-cell clones (ng/ml): IFN-γ	IL-4	IL-5	IL-10	p24 Ag produced (pg/ml)
BE PPD.1	6.55	< 0.05	< 0.05	0.2	< 5
ER.14	5.95	< 0.05	< 0.05	0.3	< 5
ER.57	8.25	< 0.05	0.3	< 0.05	< 5
AZ.43	4.27	< 0.05	< 0.05	< 0.05	< 5
VA.58	7.55	1.1	< 0.05	5.9	< 5
VMB.26	0.3	0.8	0.6	0.4	65
ER.18	1.0	1.8	8.0	7.3	98
PGB.60	< 0.2	2.0	4.5	0.1	89
PGS.69	< 0.2	0.6	2.4	< 0.05	70
MA 36	< 0.2	5.5	6.7	< 0.05	58

Established T-cell clones specific for different antigens (PPD, tetanus toxoid and purified Der p I allergen) were generated from PBMC of 3 HIV-seronegative subjects, as described in Table IV. These clones, defined as T_H1-, T_H0- or T_H2-type on the basis of their ability to produce IFN-γ and/or IL-4 were infected *in vitro* by co-culturing with irradiated HIV-infected PBMC in the presence of PHA (1%, v/v), IL-2 (20 U/ml) and polybrene (1 μg/ml). After 3 weeks, the presence of HIV DNA was assessed by semiquantitative PCR, as described (Carbonari et al. 1993). T-cell blasts (10^6/ml) were stimulated with PMA plus anti-CD3 antibody and cell-free supernatants were assessed for their p24 and cytokine production by appropriate assays (Macchia et al. 1991).

tion, possibly in association with other still unknown factor(s) released by both $CD8^+$ and T_H1-like $CD4^+$ T cells, plays a protective role against HIV replication in $CD4^+$ T cells. An alternative possibility to explain the lower efficiency of T_H1-like $CD4^+$ T-cell clones in supporting HIV replication is that $CD4^+$ T-cell clones producing only T_H1 cytokines have lower proliferation rate than clones producing T_H2 cytokines.

HIGH IgE SERUM LEVELS AT THE TIME OF SERODIAGNOSIS ARE ASSOCIATED WITH LESS FAVORABLE PROGNOSIS

If T cells producing T_H2 cytokines are more efficient supporters of HIV replication than T_H1 cells, it may be reasonable to suggest that subjects showing hyperexpression of T_H2-type responses because of genetic (such as atopic subjects) or environmental reasons (such as people living in endemic areas of nematode infections) have a less favorable prognosis for HIV infection. In order to investigate this possibility, we have recently looked at two groups of HIV-infected individuals who at the time of serodiagnosis exhibited high or low serum IgE levels, respectively. As mentioned above, high serum IgE levels would indeed be the result of increased IL-4 production. Twenty-six patients who at the time of serodiagnosis (1985) had serum IgE levels higher than 200 IU/ml (mean value 491 ± 75) were compared

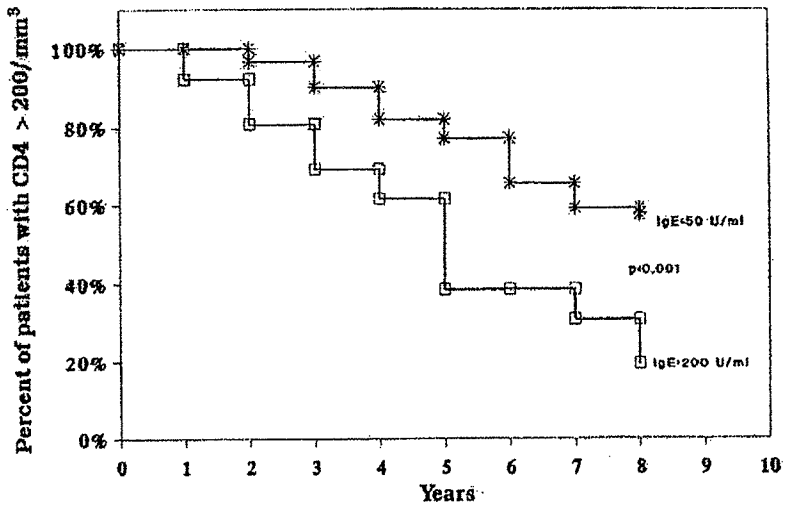

Figure 3. Different median rate of $CD4^+$ T-cell depletion in HIV-infected individuals showing normal (< 50 U/ml; n = 50) or elevated (> 200 U/ml; n = 26) IgE serum levels at the time of diagnosis of HIV infection.

with 50 patients with IgE levels lower than 50 IU/ml (mean value 18 ± 2). At that time, both groups of HIV-infected subjects had quite normal and equivalent numbers of circulating $CD4^+$ T cells (922 ± 84 and $915 \pm 48/\mu l$, respectively). Eight years later, subjects with high IgE serum levels at the time of serodiagnosis revealed a significantly greater depletion of circulating $CD4^+$ T cells than subjects with normal serum IgE levels (Fig. 3). In the group with high serum IgE levels both the number of patients with full-blown disease and of deceased patients were significantly higher (data not shown). Interestingly, no differences between the two groups of HIV-infected patients in either the initial numbers of circulating $CD8^+$ T cells or the serum IgA levels were found, suggesting that elevation of serum IgE represents an independent predictor of unfavorable prognosis.

CONCLUDING REMARKS

During the past 2 years, a very simple theory that seeks to explain the causes of the relentless and ultimately fatal decline of AIDS patients has been gaining great success. The theory holds that a patient's fate is determined by which of two types of immune effector cells, designated T_H1 and T_H2, has the upper hand in controlling the immune responses. According to this theory, HIV-infected subjects switch from a T_H1 protective to a T_H2 unprotective or even harmful state as the disease progresses (Clerici & Shearer 1993a). The T_H1/T_H2 switch hypothesis has been mainly based on measurements of cytokine production by blood cells from HIV-infected, but otherwise healthy, donors showing that IL-4 and IL-10 production increased while IL-2 production decreased during the course of infection (Clerici et al. 1993b, 1994). If correct, the theory would have major implications for efforts to develop AIDS vaccines and therapy. The idea would be to use drugs or vaccines that bolster T_H1 responses, while reducing T_H2 responses.

To prove or disprove this theory, we used a series of experimental approaches. Taken together, the results of our studies failed to confirm the hypothesis of a T_H1/T_H2 switch during the progression of HIV infection. Indeed, no increase of IL-4, IL-5 and IL-10 production was found in either short-term cultures of mitogen-stimulated PBMC or at the level of $CD4^+$ T-cell clones derived by PHA stimulation of single T cells from HIV-infected individuals compared to controls or among HIV-infected subjects in different phases of infection. Quite the contrary, a preferential depletion of IL-4-producing T-cell clones was observed in HIV-infected patients with reduced levels of circulating $CD4^+$ T cells. In the other two experimental approaches, based on the clonal expansion of skin-infiltrating T cells already activated *in vivo* or the expansion of specific T cells activated with antigen *in vitro*, respectively, enhanced proportions of $CD4^+$ (and even of $CD8^+$) T-cell clones able to produce T_H2-type cytokines were found. Again, however, even in these models no significant reduction in the proportions of T-cell clones producing T_H1-type cytokines was observed. Thus, at most, an

eventual switch from T_H1 to T_H0 (but not to T_H2) profile of cytokine production can be suggested.

One possible explanation for these findings is that the enhanced expression of T_H2 cytokines does not involve every single T-cell (all of which are expanded in the cloning system based on PHA stimulation), but is restricted to memory T cells (which are selectively expanded in both the skin and the antigen-specific models of T-cell cloning). This hypothesis is consistent with recent data from Meyaard et al. (1994), showing enhanced expression of T_H2 cytokines by T-cell clones obtained by PHA stimulation of single $CD45RO^+$ (memory) peripheral blood T cells from HIV-infected individuals. An alternative possibility is that the difference is due to the influence of APCs, which are involved in both the activation of skin-infiltrating T cells *in vivo* and the antigen presentation *in vitro*, whereas in the cloning system based on PHA stimulation of single T cells, irradiated allogeneic feeder cells from healthy subjects are used.

The role of cytokines produced by APCs in influencing both the *in vivo* differentiation of naive T cells and the *in vitro* development of memory T cells into the T_H1 or the T_H2 phenotype has been widely demonstrated (Swain 1991, Hsieh et al. 1992, Maggi et al. 1992, Parronchi et al. 1992, Romagnani 1992, Seder et al. 1992, Manetti et al. 1993, 1994). For example, we have clearly shown that the presence of IL-12, IFNα or IL-1rα in bulk culture favors the development of T_H1 clones, whereas addition of IL-4 and/or neutralization of IL-12 shift the differentiation towards the T_H2 profile (Maggi et al. 1992, Manetti et al. 1993). Interestingly, production of both IFNα and IL-12 have been found to be defective in HIV-infected individuals (Gendelman et al. 1990, Chehimi et al. 1994). Thus, it is possible that in HIV-infected subjects such a combined defect may underlie the enhanced expression of T_H2 cytokines even in response to antigens, such as PPD, which at least in healthy subjects preferentially expand $CD4^+$ T_H1-like clones. The possible role of APCs from HIV-infected individuals in favoring the development of $CD4^+$ T cells producing T_H2 cytokines is now being investigated in our laboratory.

The most interesting observation emerging from this study, however, is that T_H2- and T_H0-like T-cell clones, when infected *in vitro* with HIV, are more efficient than T_H1 clones in supporting HIV replication. Although preliminary experiments seem to suggest that a prevalent production of T_H2 cytokines favors HIV replication not only by T-cell clones infected with HIV *in vitro*, but even by freshly derived $CD4^+$ T cells from HIV-infected subjects, the relevance of these *in vitro* findings to the *in vivo* situation still remains unexplored.

The preferential HIV replication in $CD4^+$ T cells producing T_H2 cytokines, resulting in more rapid death of, and viral spread by, this cell type may reconcile different observations. First, it may indeed explain the lack of spontaneous IL-4 mRNA expression found in peripheral blood and lymph node cells from HIV-infected subjects (Graziosi et al. 1994), in spite of their probably enhanced overall

ability to produce T_H2 cytokines in response to antigenic stimulations. Second, it may explain both the reduced cloning efficiency and the preferential depletion of IL-4- (and IL-5)-producing $CD4^+$ T-cell clones derived by PHA stimulation of single T cells from the peripheral blood of HIV-infected patients examined in advanced phases of infection. Finally, it may account for the significantly higher reduction of circulating $CD4^+$ T cells that we have found in the peripheral blood of HIV-infected patients showing high IgE serum levels at the time of serodiagnosis, as well as for faster progression to AIDS of the HIV-infected Ethiopian immigrants in Israel who revealed an immune activation of T_H2-type prior to any HIV exposure (Bentwich et al. 1994, submitted). In the light of these findings, it may not be unreasonable to investigate whether atopy in developed countries and nematode infestations (e.g. schistosomiasis) in developing countries really do represent conditions for less favorable prognosis in HIV-infected individuals.

SUMMARY

Different experimental approaches were used to prove or disprove the "T_H1/T_H2 switch theory" of HIV-infection. No increase, or even a decrease, in the production of T_H2-type cytokines (IL-4, IL-5, and IL-10) by either bulk circulating mononuclear cells or $CD4^+$ T-cell clones generated by PHA stimulation of single T cells from HIV-infected individuals in all stages of disease compared to HIV-negative donors was observed. However, enhanced proportions of $CD4^+$ T-cell clones able to produce both T_H1-type and T_H2-type cytokines (T_H0 clones) were derived from either skin-infiltrating, *in vivo*-activated, T cells or *in vitro* antigen-stimulated peripheral blood T cells of HIV-infected individuals. Of note, T_H1, T_H2 and T_H0 clones obtained from HIV-seronegative healthy donors showed different ability to support viral replication after infection with HIV *in vitro*. All T_H2 and most T_H0 clones supported HIV replication efficiently, whereas T_H1 clones did not. These results suggest preferential HIV replication in T cells producing T_H2-type cytokines rather than T_H1/T_H2 switch in HIV infection.

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